SOME EFFECTS ON FUNDULUS OF CHANGES IN THE DENSITY OF THE SURROUNDING MEDIUM.

GEORGE G. SCOTT.

Bert, '71, found that certain teleosts changed in weight on being immersed in water having a different density from that of the normal medium to which they were accustomed, though in some cases death occurred before any considerable change in weight had taken place. Garrey, '05, found that 80 per cent. of *Fundulus heteroclitus* taken from sea water lived in fresh water for six weeks. Loeb, '00, said, "*Fundulus* can be thrown from sea water into distilled water without any considerable swelling or without any visible injurious effects." Sumner, '06, reported a series of experiments from which he concluded that *Fundulus* did not survive transference from sea water to fresh water. He also concluded that diluted sea water containing only 2 per cent. to 4 per cent. of the salinity of pure sea water had a salutary influence on the preservation of life. Since the osmotic pressure of fresh water is but little less than this diluted sea water and since *Fundulus* survived in this solution but not in the other, Sumner concluded that the question of survival or death is not a question of difference in osmotic pressures. Sumner also investigated the changes in weight undergone by *Fundulus* in different dilutions of sea water. He found that in fresh water there was a slight gain which was followed by a loss until near the normal weight. In general transference to a hypertonic solution resulted in loss of weight, while in hypotonic solutions there was noted a gain in weight. And yet the change in weight bore no constant ratio to the changes in the osmotic pressure of sea water. Sumner found indications of a smaller gain in *Fundulus* from Woods Hole than in those from the New York Aquarium. He accounts for this as being due to differences in the physiological state of the organism at the higher temperature of the summer months. Their more active metabolism at this period might account for the greater permeability. On account of the diverse position

taken by Garrey and Loeb on the one hand and Sumner on the other the present author desires to publish observations on the changes in weight in *Fundulus heteroclitus* resulting from immersions in solutions differing in density from that of sea water.

Fundulus heteroclitus, although found in sea water and brackish waters, is known at times to pass into fresh waters. Quoting from Sumner, p. 56, we find, "Bean, '03, says of *heteroclitus* that it sometimes ascends streams beyond tide water"—"Smith, '97, states that it is often found landlocked in ice or quarry ponds." "Dr. H. M. Smith informs me that it is found permanently in the vicinity of Washington, in the Potomac and its tributaries and also in ponds."

It is thus abundantly established that *heteroclitus* is found in fresh waters as well as brackish waters and even sea water. This does not mean however that they will readily survive sudden changes from salt to fresh water or *vice versa*. Sumner tried the effect of acclimatization by transferring 25 *F. heteroclitus* from the sea water (sp. gr. 1.025) to fresh water reducing the salinity by .001ths, hourly. At the end of thirty days but eleven were alive. From this Sumner concluded that complete acclimatization failed. Of course this is true since fourteen were dead. But what of the survivors? Why was not complete individual acclimatization exhibited in the cases of these? With regard to acclimatization Eugene Smith, '12, says that *Fundulus heteroclitus* may be transferred from salt and brackish to fresh water. They may be transferred more safely, the less degree of salinity there is in the water from which they come. Furthermore, while very few of those transferred from salt water directly to fresh survived the sudden change, an increasing number survived of those gradually transferred in the course of a week or two through a number of changes of water. My records show that such fishes lived from four to six months up to two years—one lived over three years." The above probably represents the truth of the matter. It will be observed here that the above writer emphasizes the suddenness of the transfer. The effect of a stimulus is related to the suddenness of its onset. In fact the living mechanism may be injured under too sudden as well as strong stimuli.

On July 10, 1908, I transferred ten *Fundulus heteroclitus* through a series of changes from sea water to a large rectangular dish containing about 20 liters of fresh tap water. In the bottom of the dish was placed some gravel from the shore near by—the gravel having been thoroughly washed in tap water. The water in the tank was completely changed every day or so—and once a week the tank was thoroughly cleaned. Food was placed in the tank daily, but all uneaten portions were removed after a few hours. On the whole the fishes did not seem eager for the food. Most of the fishes died during the next three or four weeks but one fish was alive and apparently in good condition on September 8, 1908, sixty days after the beginning of the experiment. A rough test of the water toward the end of the period showed a slightly greater amount of chlorine than was present in fresh water. And yet the hydrometer recorded a specific gravity of about 1.000. At first we might think that Sumner's contention as to the life-saving action of a small amount of salts was borne out here. But the amount of salts in which this specimen survived was less than that claimed by Sumner as necessary to exert a life-saving action. At the same time I do not wish to disclaim Sumner's contention. I only wish to point out a case where this conclusion does not follow.

Not only will *Fundulus* survive transfer to fresh water, but regeneration of removed tissues takes place under these conditions. This is shown by the results of the following experiments carried out with *Fundulus heteroclitus* taken from the New York Aquarium from the diluted harbor water which had a specific gravity of about 1.012 (sea water having a sp. gr. of 1.025). The caudal fin was removed in the same manner as described in a former paper by the present author (Scott). After the removal of the fin the fishes were transferred gradually to fresh water in a large rectangular jar which was constantly aerated from the compressed air supply in the laboratory at the College of the City of New York. The water was siphoned off nearly every day and replaced by a fresh supply from the tap. Fresh-water plants were kept in the experimental aquarium during the latter part of the period. The specimens were fed with fish food which they soon learned to take. I was not concerned at

the time with the question as to the percentage of *Fundulus* that survive transfer to fresh water. The experiment was begun on March 13, 1909. On April 2, twenty days after, ten survivors were removed. The total body length was taken together with the length of the regenerated tissue of the caudal fin. The average actual length of the regenerated tissue of the caudal fin was 0.238 cm. The remaining ten survivors were removed on April 16, thirty-four days after the experiment was begun. The average actual length of regenerated tissue was .495 cm. The temperature of the water was about 20° C. A second experiment was begun on November 18, 1910. Out of thirty fishes operated on and placed in fresh water on November 18, but seven survived. In forty-two days during which the fish lived in fresh water the length of regenerated tissue was .41 cm. Out of a lot of thirty other specimens operated on two weeks later six survivors on December 30, 1910, about twenty-eight days after the experiment was begun, showed a regeneration of .23 cm. During the course of the experiment the fishes were transferred every few days to a smaller glass jar and the aquarium thoroughly cleaned and replenished with fresh water from the tap. In 1908 I found that the average length of regenerated caudal fin tissue of 108 specimens of *Fundulus heteroclitus* in sea water at Woods Hole for a month was about 0.6 cm. It will be seen that in fresh water the regeneration of the first of the above lots was 0.495 cm. in 34 days, that of the second lot, 0.41 cm. in 42 days. It appears probable that in fresh water the amount of regenerated tissue is decreased but because the animal as a whole is affected by fresh water. Further discussion is not proper on account of the meagerness of the data. Results show that not only does *Fundulus* survive transfer to fresh water but also that under these conditions, physiological processes are sufficiently normal for the regeneration of removed tissue.

Sumner in his practice in using numbers for fishes, found that frequently individuals died and so the carrying on of the experiment was interfered with. I determined therefore to study the changes in weight in *Fundulus* in solutions differing in density from that of sea water, by keeping a record of the individual changes in weight. Now since some specimens of *Fundulus* die sooner than

others when transferred from sea water to fresh water, and since an increase in weight occurs when they are thus treated, the idea occurred to me as to whether the change in weight is less for the surviving individuals than in the case of those dying. Accordingly a number of experiments were tried with individuals transferred to separate dishes each containing a liter of fresh water. Each specimen was rinsed in fresh water, the free water absorbed on a soft clean towel, the specimen weighed and then placed in the dish indicated above. After a certain period the specimen was again similarly weighed and replaced in the dish with fresh water again. As a check a number of specimens were reweighed immediately. The dish in which they were weighed was also weighed after the fish was removed and compared with its former weight. No fish was handled more than was absolutely necessary. In fact handling was limited to picking the fish from the towel, placing it in the experimental dish.

Difference of opinion exists as to the effect of removing the scales or injuring the skin. For example, Bert found that the removal of mucus from the skin of the eel caused its death in sea water—where it would otherwise survive this transfer. He found that the eel survived transfer from fresh to salt water and was surprised when in a similar experiment carried out by his assistant, the eel died. He learned that the assistant had unconsciously removed mucus from the skin of the eel in the struggles involved in making the transfer. The experiments of Garrey corroborated Bert. Removing the scales or skin from portions of the surface of the body resulted in the rapid death of *Fundulus heteroclitus* on being transferred to fresh water, or to sea water, but of those kept in diluted sea water, approximately isotonic with the blood, none had died at the time the others were dead. Sumner obtained opposite results for in an experiment which he carried out at Woods Hole; he transferred *Fundulus heteroclitus* to sea water full strength or to diluted sea water having a sp. gr. of 1.001. He found that most lived although the skin had been removed from one entire side of the fish. In fresh water all these fish were dead in a few days. Sumner called attention to the well-known fact that hardy species survive mutilations of the body surface. In fact in experiments which

he carried on at the New York Aquarium he noted the beginning of regeneration of scales six days after they had been removed, and during which they had been kept in sea water with a specific gravity of 1.025 having been transferred to this from the dilute harbor water with a density of 1.007. Mr. Denyse, of the New York Aquarium, informs me that it has been his experience that death usually accompanies serious injury to skin of fishes. Notwithstanding the difference of opinion cited above it is true nevertheless that in none of the experiments about to be described were scales removed. In fact, there was no visible evidence of the coat over the scales being injured. Attention is here called to a further consideration before the experiments are described.

The specimens used in the following experiments bore no surface abrasions and to the eye appeared normal. They were taken as needed from a large storage tank supplied with running sea water to which tank new specimens were added by the collector from time to time. Not only was a rough selection employed here but a further examination was made before they were used in the experiment. 153 specimens were used in 21 experiments and 988 weight determinations were made.

Control experiments with *Fundulus* in sea water showed on the whole a slight loss in weight as time went on. The results were similar to those found by Sumner. Certain individuals died in this control experiment showing that death in the following cases is not always due to the experimental solutions. In an experiment with six fishes in which an individual record of each was kept the entire lot was dead about eighteen hours after the experiment began. Variations in the individual weights were evident. But since they were all dead at the end of the period in question the average results will alone be given. Thus the average gain in weight at the end of two hours was 5.2 per cent.; at the end of five hours, 8.7 per cent.; at the end of eighteen hours, 15.7 per cent. In a second lot, the results were similar, five of a lot of six specimens were dead at the end of twenty hours. The change of weight was as follows, at the end of three hours 4.4 per cent.; at the end of five hours, 7.3 per cent.; at the end of seven hours, 9.6 per cent. This is the average of five specimens. The sixth was dead and showed a gain of 12.4 per cent. The

average of the other five, all dead at the end of twenty hours, was an increase in weight of 19.0 per cent. In a third experiment it was clear that an increase in weight was greater in some specimens than in others. While all were dead at the end of twenty-three hours, yet three of the nine experimented with were dead at the end of nine hours. Three others appeared "sick"; they would swim on the side, turn over, follow the bottom, then start to swim again. The gain in weight in these was above that of the others still alive. In other words the results of these three experiments indicated that where there was an excessive gain in weight this was followed by early death. A comparison with these results with those of a fourth experiment about to be described in detail demonstrates the difference in different lots of fishes which in external appearance seemed normal. In this fourth experiment lasting over two days during which eleven weight determinations were made of nearly every specimen this differentiation was all the more striking and because of this, the experiment will be described in more detail.

The actual weights are not given. The table gives the percentage change in weight at each period which is found by comparing the weight of each specimen at the end of the period in question with the original loss in weight. The sign "+" means a gain in weight, while the sign "−" means a loss in weight. The results of this experiment are shown in Table I. The first noticeable feature in this record is the individual variation in the change in weight in all of the specimens. At the end of the observational period seven are dead and eleven are alive; while one is recorded as being lost. All of those alive at the end of the experiment weigh less than they did at some period after the beginning of the sojourn in fresh water. Indeed five weigh less than they did at the beginning of the experiment. If the changes in individual weight be followed, it will be seen to be a case of ups and downs in weight increases. All the specimens show a gain in weight during the first period. In some the gains are greater than in others. Specimen 5 with an initial gain of 8.0 per cent. recovers from this and later loses in weight and is alive at the end of the period. Specimen 18, on the other hand, shows a gain of 2.1 per cent. at first but this increases steadily

Table I.

Showing the Percentage Change in Weight in Individual *Fundulus heteroclitus* at Different Times During Immersion in Fresh Water.

Periods.	1	2	3	4	5	6	7	8	9	10	11	
Hours.	1+	3	4	18+	20+	22+	24	40	46	47	54+	
1 =	+3.1	+5.2	+5.0	+2.7	+5.6	+3.1	+3.8	+0.4	+1.4	+2.7	−2.9	(alive)
2 =	+2.2	+3.5	+0.4	+0.9	+0.3	+1.6	+1.8	+0.5	+0.9	+3.5	+0.3	"
3 =	+1.5	+1.9	+2.3	+1.9	+2.6	+2.4	+2.0	+0.9			+1.5	"
4 =	+2.9	+4.8	+4.5	+4.1	+3.7	+4.8	+4.4	+1.7	+5.7	(lost)		(lost)
5 =	+8.0	+9.5	+6.6	+7.8	+6.3	+8.1	+8.3	+3.0	+2.7	+1.4	−1.4	(alive)
6 =	+2.7	+4.3	+5.4	+7.1	+5.6	+6.4	+5.6	+3.1	+5.4	+5.6	+12.1	(dead)
7 =	+3.3	+4.0	+0.9	+4.3	+2.3	+1.5	+0.8	+1.0	+2.9	+3.8	−5.8	(alive)
8 =	+2.3	+3.6	+2.2	+1.2	+3.9	+1.0	+2.2	+1.2	+1.7	+3.7	+2.7	(alive)
9 =	+1.7	+2.7	+1.1	+1.2	+2.2	+1.9	+2.1	+1.2	+0.3	+3.9	+3.7	(alive)
10 =	+1.0	+3.4	+9.3	+14.1	+17.0	+17.8	+20.9	+28.0				(dead)
11 =	+2.3	+3.3	+8.6	+11.5	+15.2	+17.5						(dead)
12 =	+3.7	+4.3	+1.1	+9.2	+10.3	+12.0	+18.7					(dead)
13 =	+1.8	+2.7	+2.8	+2.1	+4.3	+1.4	+2.7	+2.3	+1.4	0	+0.5	(alive)
14 =	+1.9	+3.4	+4.9	+4.0	+5.7	+4.5	+4.6	+3.4	+2.7	+1.5	+0.2	(alive)
15 =	+1.4	+3.3	+2.0	+2.7	+2.9	+3.1	+2.0	+1.1	+2.0	+1.4	−9.6	(alive)
16 =	+4.4	+3.4	+6.1	+7.6	+8.0	+9.9	+9.2	+20.2				(dead)
17 =	+2.0	+3.4	+2.9	+2.7	+3.0	+3.4	+2.9	+3.3	+4.2	—	+16.4	(dead)
18 =	+2.1	+4.0	+8.0	+8.7	0	+10.3	+10.6	+16.7				(dead)
19 =	+5.1	+2.8	+1.2	+0.3	+0.7	+0.4	+0.2	+1.6	+2.7	+3.5	−4.9	(alive)

until it is dead at the end of the forty-hour period having shown a gain of 16 per cent. It is well known that fishes absorb water and increase in weight after death. Some of the final weight increases in the dead specimens may have been due to post-mortem processes. But there is ample evidence that the increase in weight began before death. This is shown by specimens 10, 11, 12 and 18. On the whole, selection takes place when *Fundulus* is placed in fresh water. This experiment shows at least one phenomenon accompanying the acclimatization of *Fundulus* to fresh water. As was said above, it is now well known that when *Fundulus* is put in fresh water from sea water, that some die and some survive. The present experiments show that the survivors after the first gain weight incidental to being placed in fresh water, recover from this gain, lose weight and survive. Others either at the beginning or later begin to steadily increase in weight. This may be sudden and at any time during the course of the experiment. In another experiment, where *Fundulus* was placed in a solution of one half sea water plus one half fresh water, some specimens gained somewhat as the survivors in the present experiments. They later lost. Others showed a slight loss from the beginning. Those that died resembled those that died in sea water, that is, the changes in weight gave no clue to the cause of death. The effect of this diluted sea water is not one half the effect of the sea water. The osmotic pressure of the diluted sea water is about half that of full strength sea water. In other words the effect of the two solutions is not proportional to the differences in their osmotic pressures. The result is similar to that found by Sumner, '05. Some of the specimens in the diluted sea water died but showed no peculiar weight differences.

Finally individual records were kept of changes in weight when fishes of this same species were placed in sea water to which known quantities of sea salt had been added. These experiments also revealed great variations in weight of one individual as compared with another. As would be expected the survival time decreased as the strength of the solution increased but not in proportion to the increase in the density of the surrounding medium. The results of some of these experiments are shown

in Table II. The specimens lost in weight in these hypertonic solutions. The fishes in solution "D" are dead in four hours and lost but 9.5 per cent. of their weight while the fishes in the less dilute solutions "A," "B," "C" show a much greater loss

Table II.

A = Showing the Changes in Weight in ***Fundulus*** in a Solution of Seawater to Each Liter of which was added 16 Grms. of Sea Salt.

Periods.	1	2	3	4	5	6	7	8	9
Hours.	3+	7+	22+	27+	43+	54+	67	91	118+
1 =	−6.3%	− 7.6	−15.4	−14.8	− 9.7	−13.7	(dead)		
2 =	−9.6	−16.1	−14.4	(dead)					
3 =	−2.7	− 6.2	− 3.4	− 3.4	− 1.6	− 1.8	−1.3	−2.0	−0.9
4 =	−3.3	− 5.3	− 3.7	− 4.0	− 8.6	− 8.6	(dead)		
5 =	−4.2	− 4.9	− 9.7	− 8.7	−16.5	(dead)			

B = Changes in Weight in Solution of Seawater to Each Liter of which 22 Grms. of Sea Salt was added.

Periods.	1	2	3	4	5
Hours.	3+	6+	8+	22+	
1 =	− 9.4%	−12.6	−13.2	−17.9	(dead)
2 =	− 9.6	−13.7	−14.9	−19.3	"
3 =	− 6.0	−17.7			"
4 =	− 9.9	−14.7	−16.1	−17.0	"
5 =	−10.0	−13.6	−13.9		"

C = Changes in Weight in Solution of Seawater to Each Liter of which 42 Grms. of Sea Salt is added.

Periods.	1	2	
Hours.	3+	6	
1 =	−12.4%	−15.5	(dead)
2 =	−12.5	−15.9	"
3 =	− 9.7	−13.4	"
4 =	− 8.4	−11.0	"
5 =	−11.8	−17.6	"

D = Changes in Weight in Solution of Seawater to Each Liter of which 62 Grms. of Sea Salt was added.

Periods.	1
Hours.	4
1 =	− 9.6% (dead)
2 =	− 8.1 "
3 =	− 7.9 "
4 =	− 8.7 "
5 =	−12.5 "

in weight and after a longer time. This rather sudden death of these in solution "D" accompanied by a smaller loss in weight as compared with the others suggests that in these hypertonic experiments death may be due to the direct action of the excessive amount of sodium chloride present in solution. Still there are evidences of the ability of the organism to react against even this condition for in one experiment in which the fishes were kept in a solution of sea water to which 16 gms. of sea salt were added per liter, most of the specimens were dead after two days showing a loss in weight. One (no. 3) lived for nearly five days and showed in its weight determinations fluctuations similar to the survivors in fresh water although of course in this case they were of an opposite nature due to the hypertonic solution. Mather, '81, suggested that the cause of death of salt-water fishes in fresh water was not due so much to chemical differences as to differences in osmotic pressures. Sumner takes the opposite view. But both may play a part in producing the results. It is possible that *Fundulus* resembles the eel, *Anguilla*, and undergoes a reduction of the osmotic pressure of its blood after sojourn in fresh water. Dakin, '08, found that in sea water the blood of *Anguilla* resembled that of marine teleosts, though not quite as great. In fresh water, the osmotic pressure of its blood was similar, though not quite as low as that of the blood of fresh-water teleosts.

Sumner found that a loss of salts took place when *Fundulus* is placed in fresh water and that the amount lost decreased as the time of sojourn in fresh water increased. This result also indicates that a decrease in the permeability of the limiting membranes of the body takes place due to the change in the environment.

At least two positions can be taken with regard to this matter. In the first place however the question arises as to the part of the body concerned in the effects noted. Sumner gives experimental evidence based on studies with teleosts to the effect that the gill membranes are the structures concerned. Experiments of my own with elasmobranchs show the same results. With regard to the two possible views to take, one is, that the kidneys regulate the osmotic pressure of the blood when the organism is immersed in fresh water, eliminating the excess of water taken

in through the gill membranes. But what of the constant effect of this excessive work on the kidneys in the case of specimens successfully transferred from sea water to fresh and living for a number of years in fresh water, as Smith has described. Such a view is manifestly weak. There is but little doubt that the changes brought about in the organism due to immersion in fresh water are due to diffusion of water through the gill membranes, and that the permeability of the gill membranes is changed. A second view as to what subsequently happens is that the gill membranes after this attack are modified. And in such a process must not other parts of the organism be concerned? The gill membranes cannot be changed of themselves. They are under the influence of the nervous system. Blood carries materials to the membranes. The experiments show analogies to the response of animals to the inoculation of bacteria, a period of ups and downs, recovery in the case of some, death in others. It seems to me that views which place the emphasis upon the influence of the external agents on the membranes and explain acquired impermeability as being due to some sort of tanning action, are incomplete. What kind of respiration could take place through tanned membranes? Evidence has been obtained from the plant world to the effect that plant cells can modify their permeability. It is not necessary, however, to agree with Philip, '10, when he says, "This fact and others which have just been quoted, will serve to show that a purely physical theory of the exchanges which take place across a living membrane is inadequate; there is a physiological permeability as well as a physical permeability." This is but begging the question.

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